ENDOCRINE DISRUPTORS: MODELING THE INTRACELLULAR RESPONSE

Michael Breen, Rory Conolly

National Center for Computational Toxicology, U.S. EPA, Research Triangle Park, NC, USA

ABSTRACT

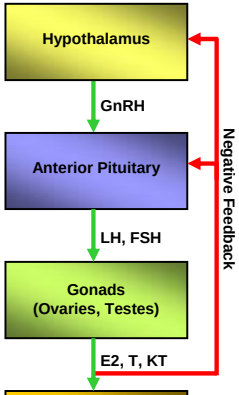
Scientists have identified alterations in the concentration dynamics of specific hormones as risk factors for common cancers such as breast cancer (estrogen, progesterone), endometrial cancer (estrogen), and prostate cancer (estrogen, testosterone). These adverse hormonal changes in the tightly regulated endocrine pathways can be induced from exposure to exogenous endocrine disruptors. Chemicals capable of acting as endocrine disruptors are ubiquitous with environmental sources that include household detergents, pesticides, plastics, pharmaceutical estrogens, industrial chemicals, and byproducts of incineration, paper production, and fuel combustion. Ecological exposures to endocrine disruptors are primarily from industrial and waste water treatment effluents, while human exposures are mainly through the food chain. The adverse effects induced by exposure to endocrine disruptors can be mediated through alterations in the enzymes involved in steroid synthesis. We are developing a mechanistic mathematical model of the intratesticular and intraovarian metabolic network that mediates steroid synthesis to describe the dose-response for endocrine disruptors, and to identify and link new robust molecular biomarkers of exposure that are indicative of the ultimate adverse effects. The deterministic model describes the biosynthetic pathways for the conversion of cholesterol to the sex steroid hormones (estradiol, testosterone, and 11-ketotestosterone) secreted by the testes in fish. The model includes the intermediate metabolites and enzymatic reactions for the multiple pathways involved in the biosynthesis of the sex steroids. Changes in the concentration dynamics of the secreted hormones are used as an index of the endocrine disruption. The initial concentrations and enzyme kinetic reaction rates were taken from the literature or set to biologically reasonable values. This mechanistic model allows for an improved understanding of the source-to-outcome linkages and dynamic dose-response behavior at the molecular level for effective use of biomarkers for risk assessments with endocrine disruptors, including their possible effects on endocrine-induced cancers. Since the biosynthetic pathways for the sex steroids are evolutionarily conserved to a significant extent, this model is likely to also be relevant for mammalian species.

LINKING BIOMARKERS OF EXPOSURE TO EFFECTS

	Molecular	Cellular	Organ	Individual	Population
Biological Effects	Receptor-ligand interaction, DNA binding, enzyme activity	Altered signaling, gene activation, protein synthesis	Altered physiology and tissue morphology	Impaired development and reproduction, cancer, death	Structure, Extinction
Biomarkers	mRNA, protein, enzyme levels	Metabolite profiles	Functional and structural change (pathology)	Altered reproduction or development	Decreased number of animals
Computational model	Systems biology models				
Small fish model	Fathead Minnow Partially characterized genome High ecological/regulatory relevance Molecular markers, metabolomics				

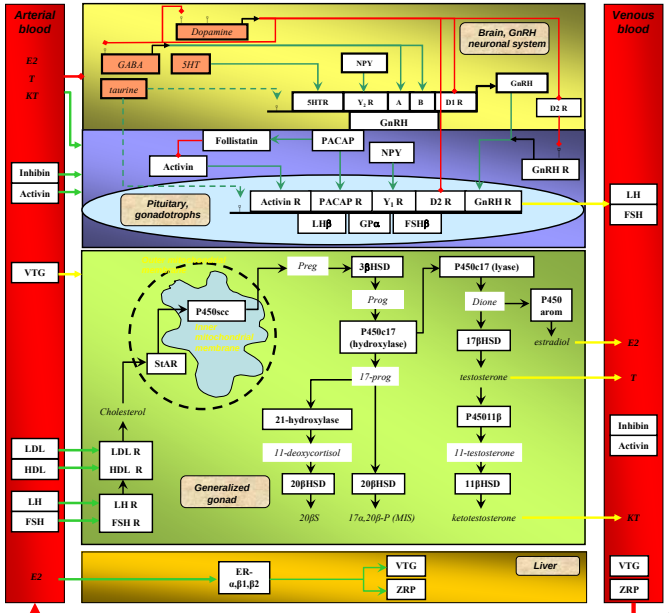
HYPOTHALAMIC-PITUITARY-GONADAL (HPG) AXIS

HPG Axis



Feedback control system of the HPG axis that regulates synthesis and secretion of primary steroid hormones (estradiol (E2), testosterone (T), and 11-ketotestosterone (KT, only in male fish)) by the release of gonadotropin releasing hormone (GnRH) from the hypothalamus, and luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary.

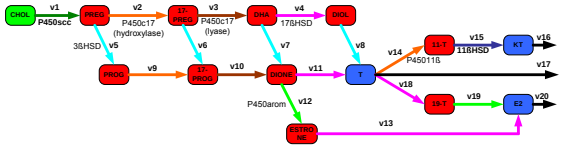
Conceptual Systems Model



Conceptual systems model shows key regulatory components of HPG axis. Green and red arrows indicate activation and negative feedback (inhibition), respectively. White boxes indicate proteins and peptides. Small molecules (e.g. steroids and neurotransmitters) are shown in italics.

COMPUTATIONAL MODEL

Intratesticular Steroidogenic Pathway



Deterministic Model

$$\frac{d}{dt}CHOL = -v1 \qquad \frac{d}{dt}PROG = v9 - v5 \qquad \frac{d}{dt}E2 = v13 + v19 - v20$$

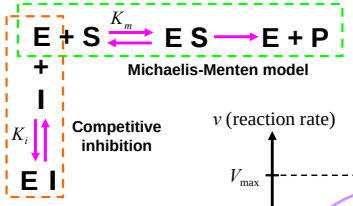
$$\frac{d}{dt}PREG = v1 - v2 - v5 \qquad \frac{d}{dt}17PROG = v6 + v9 - v10 \qquad \frac{d}{dt}19T = v18 - v19$$

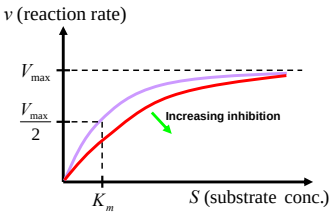
$$\frac{d}{dt}17PREG = v2 - v3 - v6 \qquad \frac{d}{dt}DIONE = v10 + v7 - v11 - v12 \qquad \frac{d}{dt}11T = v14 - v15$$

$$\frac{d}{dt}DHA = v3 - v4 - v7 \qquad \frac{d}{dt}ESTRONE = v12 - v13 \qquad \frac{d}{dt}KT = v15 - v16$$

$$\frac{d}{dt}DIOL = v4 - v8 \qquad \frac{d}{dt}T = v11 + v8 - v14 - v17 - v18$$

Enzyme Kinetics





Mathematical Model

$$v = \frac{V_{\max} S}{S + \alpha K_m} \qquad \alpha = 1 + \frac{I}{K_i}$$

3 parameters: $V_{\max}, K_m, K_i$

EDC EXPOSURES

Small fish exposure system

- Exposure of male and female fathead minnows to EE2 (synthetic estrogen): high ecological/regulatory relevance
- Dose levels: 0 (control), 10, 100 mg/L
- Dosing phase: 8 days
- Recovery phase: 8 days
- Tissue sampling: day 1, 4, 8, and 16

Fathead minnows

PARAMETER ESTIMATION

Objective function: $f = \sum_{i=1}^{I}\sum_{n=1}^{N}\left[S_{i,n} - S_i(t_n, \theta)\right]^2$

where: I = number of species (metabolites)

N = number of time samples

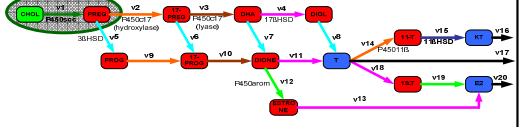
S = concentration of species (metabolite)

θ = adjustable model parameters

- Apply an iterative optimization algorithm
- Simultaneously estimate parameters for all dose concentrations

MODEL SIMPLIFICATION

Rate-limiting reaction



- Motivation
  - More intuitive understanding of dynamic functional behavior
  - Reduces number of parameters
- Method
  - Identify rate limiting step(s): quasi-steady state approximations
  - Identify preferred pathways

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DISCLAIMER

This work was reviewed by the U.S. EPA and approved for publication but does not necessarily reflect Agency policy.

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